STUDIES ON INSECT SPERMATOGENESIS.

I. The History of the Cytoplasmic Components of the Sperm in Hemiptera.

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Introduction.

No one who has noted the trend of cytological study during the last twenty years can fail to be impressed with the contrast between the progress that has been made in the analysis of the nucleus, and more particularly of the chromosomes, and the relatively doubtful outcome of parallel investigations on the cytoplasm and its formed elements. Perhaps this is because no great, illuminating hypotheses have succeeded in doing for the cytoplasmic structures what the theories of heredity and sex have accomplished for the chromosomes. At all events, it is evident that we are still on very uncertain ground in regard to many questions connected with the cytoplasmic constituents, especially such as relate to the physiology and reproduction of the cell. It is the purpose of this paper to deal with certain of these problems presented by the formed elements of the cytoplasm, more particularly the mitochondria and the Golgi apparatus.

In the case of the cytoplasmic structures, as in that of the chromosomes, the maturation period of the germ cells and the subsequent phenomena of fertilization offer the best setting for a study of many problems of the cell. Especially is this true of spermatogenesis, where the growth period of the spermatocytes and their subsequent division and differentiation into the sperms afford material for a critical study of a variety of cellular structures. The activities of the various elements of the germ cells take on an added interest when one considers that the result is to be the formation of sperm which must carry the total hereditary complex of the male, locked up with a mechanism for active

locomotion and for initiating the cleavage of the egg. It follows, therefore, that if we are rightly to estimate the rôle of the sperm in fertilization we must first gain a correct conception of the processes leading to its formation. What structures are present in the germ cells? How are they distributed to the spermatids? What is the rôle of each in the differentiation of the spermatozoön? It is from the standpoint offered by such questions that these studies have been undertaken. It was originally intended to publish the results in one complete paper, but the delays incident to printing at the present time have made it seem preferable to publish them now in briefer form, to be followed later by a more detailed account. This paper contains, therefore, a summary only of the more important points, especially such as bear more immediately on certain general cell-problems.

For this study the insects offer many obvious advantages, not the least of them the fact that the history of the nucleus has been so thoroughly studied in this group that a trustworthy outline is already at hand upon which to work out further details. I have accordingly drawn the material for this study from Hemiptera belonging to the Family *Pentatomidæ,* of which the so-called " stink-bugs " are common examples. Of many forms examined, I have made use particularly of *Murgantia histrionica* Hahn, and *Euschistus euschistoides* (= *fissilis*) Voll. Other species of *Euschistus* and several other genera (*Arvelius, Brochymena* and *Nezara*) have also been used in cases where some particular point was presented with unusual clearness or afforded interesting comparisons. Among these various genera minor differences occur, but in general the phenomena are essentially the same, the factor deciding the use of one or another genus for a given purpose being usually the size of the cells.

Much of the work published heretofore on the formation of the sperm has suffered, obviously, from an insufficient technical treatment. The extensive use of acetic acid in the fixative has been the chief source of failure, as Gatenby has recently insisted, for it has turned out that certain important cytoplasmic structrues—mitochondria and Golgi apparatus—are both of lipoid nature and hence easily destroyed by fat-solvents. I have tried to obviate this difficulty by recourse to a very extensive variety

of fixing and staining reagents, a review of which will be given hereafter. For the present purpose, a few general notes will suffice.

For the purely topographic study of sperm formation, and for working out the finer details connected with the transformation of the nucleus into the sperm head, strong Flemming followed by Fe-hematoxylin and any desired counter stain, is still the most satisfactory technique. For the mitochondria I have tried a number of methods, but by far the best for most purposes is Benda's well-known alizarin-crystal violet stain for which *Euschistus* has proven rather favorable material. For the Golgi apparatus I have tried all the better known methods, including those of Cajal, Kopsch, and Gatenby, with all of which results more or less satisfactory were obtained. None of them, however, proved of any value in studying spermatogonia and early spermatocyte stages, and they were almost equaly defective for the seprmatocyte divisions. Fortunately, in the course of my experiments I found a modification of the Kopsch method which proved to be very selective in its action and made possible a complete study of the Golgi apparatus in the earliest stages, and also throughout the maturation divisions. A description of this method has already been published (Bowen, '19), to which reference may be made for the details. In subsequent statements, I will refer to this method as " modified Kopsch."

I would like to take this opportunity of acknowledging my great indebtedness to Professor E. B. Wilson, who has placed at my disposal his many preparations of Hemipteran germ cells, and whose suggestions and kindly criticism have been of invaluable assistance to me in the preparation of this paper. I am indebted also to Dr. Franz Schrader and Mr. S. C. Dellinger for assistance in the collection of material; to Mr. H. G. Barber who has identified much of my material; and to Miss Helen Daniels who has made careful copies of my original figures for the purpose of this publication.

OBSERVATIONS.

I. *Structure of the Testis and Stages of Spermatogenesis.*

The progress of spermatogenesis may be followed most easily by dividing the process as a whole into a series of more or less

arbitrary steps or stages, based for the most part upon the condition of the chromatin in the nucleus. In the case of the Hemiptera the nuclear changes have been studied with exceptional thoroughness particularly by Wilson and Montgomery, and the steps from spermatogonium up to a late period in sperm formation are completely determined. I will, therefore, make use of the descriptive terms employed by Wilson ('12) in his outline of the stages, since they furnish well known landmarks and moreover designate periods of profound nuclear changes which, it is interesting to note, are accompanied by simultaneous changes in the cytoplasm.

(a) Polymegalous Spermatocytes and Sperms in the Family Pentatomidæ.

Hemiptera of the Family Pentatomidæ possess two compact testes, each enclosed in a delicate sheath of connective tissue, which is continued into the body of the gland in the form of septa or partitions dividing it into a number of compartments or lobes. The latter are arranged parallel to the long axis of the testis and vary in number (in the forms which I have examined) from three to seven, the number being constant, however, for any particular species. Speaking generally, the plan of the testis is not unlike that of some other families of Hemiptera, but in its details there is one notable difference in respect to which this particular family is unique, so far as known. I refer to the curious fact, to which Montgomery ('98) first called attention, that in Euschistus certain lobes of the testis are composed characteristically of spermatocytes unusually large or unusually small ("dimegaly") as contrasted to those of other lobes, which latter for want a better term might be called "normal" in size. Subsequently Montgomery ('10) published a much more complete account of this unusual condition, establishing the fact that in Euschistus sp., two of the lobes consist of unusually large spermatocytes, a single lobe of unusually small ones, while the remaining three lobes are normal. He found the spermatogonia and early spermatocytes to be all of the same size, but that following the synaptic period the size differences are established by differential growth. The relative differences he found to be

always the same in any given lobe, all the spermatocytes in a lobe being equally affected. There are accordingly three distinct sizes of spermatocytes which give rise to three proportionally different spermatids and these in turn to three kinds of sperms with clearly marked morphological differences. But in the whole series the amount of chromatin seemed to be identical, the chromosome sets of the maturation divisions being similar in every respect. Montgomery examined a number of related genera but found no such size differences, or at least no conspicuous ones.

My attention was first attracted to this problem by the discovery that *Murgantia histrionica* also exhibits size-differences similar to those of *Euschistus* but much less conspicuous, two lobes having large and three lobes normal-sized spermatocytes. The expected two kinds of spermatids and sperms (Figs. 34 and 35) were also found. These facts aroused the suspicion that "dimegalous sperm" were by no means unique to *Euschistus* as Montgomery had supposed, and accordingly it occurred to me to make a survey of the whole Family *Pentatomidæ* with respect to this particular point. The results fully confirmed my suspicion, for of thirty-seven species examined, twenty showed visible size differences, sometimes of three classes, sometimes of two. Furthermore, every degree of relative difference was found, ranging from the inconspicuous difference in *Murgantia* up to a most remarkable difference in *Arvelius,* where the whole testis is dominated by the large generations of spermatocytes. I found, further, that every part of the cell shares in this differential growth except the chromosomes—the nucleus, cytoplasm, plasmosome, mitochondria, Golgi apparatus, acrosome, centrioles, spindle and chromatoid body being of a size roughly proportionate to that of the cell as a whole. It might be supposed that the morphology of the cytoplasmic constituents would be affected by these size differences, and indeed some differences do occur, but they appear to be of quite secondary importance. Finally, I have found that the processes by which the sperm head is differentiated differ rather strikingly in the large and small cell generations, as will be indicated.

Concerning the significance of these facts, we are at present quite in the dark, nor do we know that the "polymegalous"

sperms are of equal value in fertilization. I have nevertheless found these size differences of great practical value in cytological studies, for one has at his command on the one hand a complete series of stages in miniature, and on the other a selection of the same stages reproduced on a larger scale. In the description which follows I shall refer to the dimegalous cells simply as "large" and "small," since the unusually small generation presents no points of special interest.

II. *Stages Preparatory to Sperm Formation.*

The history of the nucleus from the spermatogonial cell through the close of the second maturation division has been so minutely studied in the Hemiptera (see more particularly Montgomery ('11) and Wilson ('12)) that further description would be superfluous. I will accordingly proceed at once to a consideration of the formed elements of the cytoplasm with which I have been more especially interested.

A brief reference must first be made to the centrioles, since a knowledge of their behavior is essential to a proper understanding of other cytoplasmic phenomena. At the close of the final spermatogonial division the cells are so small and crowded that the centrioles can not be identified. They may first be made out with certainty in the spermatocytes during the growth period and, when once relocated, can easily be followed through to the close of the second maturation division. The point to be noted is that when the centrioles reappear they are already located on opposite sides of the nucleus in position for the formation of the first maturation-spindle. Furthermore, the nuclear membrane bulges out and the cell wall is drawn in opposite each centriole (Fig. 11), so that the centrioles touch (or nearly touch) the wall of both nucleus and cell, the cytoplasm in the neighborhood of the centrioles being thus greatly restricted. A section through both centers accordingly gives a very characteristic picture (see Montgomery ('11), Fig. 63).

Of the remaining cytoplasmic constituents, the mitochondria are the best known and have already been described in *Euschistus* by Montgomery ('11). His account was, however, based on faulty material and I have found it necessary to make a reex-

amination of the whole subject. The following abridged account has been drawn from *Euschistus euschistoides*.

The earliest spermatogonia which I have studied are from the cysts of large pyramidal cells arranged in a rosette, (see Montgomery ('11), Fig. 3), and in these cells the mitochondria are aggregated into a dense mass fitted closely to the nuclear membrane like a cap. The position of the latter with reference to the long axis of the cell is variable, and thus no constant polarity is visible. From this point through to the diplotene stage the mitochondria are always thus massed together (Fig. 1) except during the spermatogonial divisions when they spread throughout the cytoplasm in what appears to be a tangle of exceedingly delicate threads and granules. Montgomery supposed the mitochondrial cap to be an idiosome (idiozome), and he concluded naturally that there were no mitochondria in the spermatogonia—or at least that they must be chemically different from those of later stages. In this he was clearly misled by inadequate technique. In point of fact, beginning at least with the rosette cells the mitochondria may be traced through to the mature sperm without a break in continuity or any fundamental changes in staining reaction.

With the diplotene the spermatocytes begin to increase markedly in size, and the cap of mitochondria likewise undergoes a corresponding growth (Fig. 3). At the same time it grows looser in texture and in the large cells is seen actually to consist of a tangle of fine threads. As the growth period is entered upon, the threads begin to migrate out into the general cytoplasm, and in a short time become distributed uniformly throughout the cell (Fig. 5). This movement is accompanied by an increase in the diameter of the threads and probably in their length, but their actual number seems to be little if at all affected. In the small cell generations the breaking up of the cap is somewhat different in detail, but the final result in all the cells is the same—the mitochondria are distributed throughout the cytoplasm as a mass of threads in what appears to be a hopeless tangle. This condition continues throughout the growth period—except in the large cells, where the threads may undergo more or less extensive granula-

tion—the mass of threads enlarging with the general growth of the cell as a whole.

With the prophases is inaugurated a most interesting series of events to which I would like to call special attention. In this particular period, including the prophases of the maturation divisions and also the divisions themselves, the essential phenomena are the same in both large and small generations; but the cell pictures differ somewhat in the fact that the mitochondria in the small cells are in general thread-like, while in the large cells they are in the form of granules and rather thick rods of no great length. The following account is based on the small cells, brief details of the large ones being added by way of comparison.

The early prophases of the first maturation division present nothing of special interest. Montgomery's figures of the mitochondria in this stage are essentially correct, except that the threads appeared to him very much thinner than is the case when the Benda stain is employed. In the middle prophases a change in the arrangement of the threads becomes obvious though its exact nature is difficult to state. Occasionally in a favorable cell it is possible to make out a number of ends of threads directed toward a centriole, but at this time one would certainly never guess the nature of the ultimate arrangement of the threads of which these uncertain phases give the first inkling. In the final prophases the rearrangement of the threads is completed and the nature of the whole process becomes entirely clear. As now appears, the tangle in which the threads seemed to be at the close of the growth period was much more apparent than real. The result of the rearrangement of the threads is best seen in sections containing one or both of the centrioles which are now located at opposite sides of the nucleus, thus marking out the long axis of the future spindle. Hence it is convenient to anticipate the spindle and speak of polar and lateral aspects of the spermatocyte, in the sense in which those terms are customarily applied to metaphase figures of a dividing cell. In a lateral view of the late prophase (Fig. 11), the arrangement of the mitochondria is very characteristic. In the cytoplasmic zone immediately surrounding each centriole the mitochondria are entirely absent, the

limit of their distribution being abrupt and constant. Thus the mitochondria come to form a broad, equatorial girdle enclosing the nucleus, and, as subsequent developments show, the spindle, while the centrioles occupy the open ends of the sheath-like girdle. Furthermore, the zone of cytoplasm around the centrioles is delimited by the ends of threads, of which a few can be seen in lateral view—the main bulk of the threads being lost in an equatorial tangle impossible of analysis. The actual arrangement in the vicinity of the centrioles is rendered much clearer by a study of polar views or of sections cut obliquely through one centriole. In direct polar views (Fig. 12), especially if the cell is somewhat flattened, the paired centrioles are seen to occupy the center of a cytoplasmic area which in preparations properly fixed is seen to be the area toward which the astral rays are converging. Around the edges of this clear area are arranged the ends of the mitochondrial threads which radiate out toward the equator of the cell like so many meridians of longitude. In oblique sections through one polar area (Fig. 13), the polarization of the threads toward the centrioles is very clear, and in such views one can generally follow the individual threads for some distance. It appears that the threads are quite variable in length, some being relatively short while others are long, but generally speaking any one thread seems to be oriented toward a centriole at one end only, while the free end becomes more or less bent and twisted and is lost in the tangle which encircles the equator of the cell. In cross sections through the subequatorial region, one sees for the most part only the ends of threads. It is clear from the foregoing that the maze of threads in the spermatocytes is untangled during the prophases, apparently under the influence of the centrioles, resulting finally in the polarization of most (all?) of the threads toward the opposite centrioles, while in the equatorial zone the free ends of the threads apparently become lost in an inextricable tangle.

Meanwhile the nuclear membrane fades out, the chromosomes take up their position on the spindle and the metaphase figure of the first maturation division is complete (Fig. 14). In sections through the long axis of the spindle numerous threads can be made out directed toward the centrioles, but in polar view the orientation of the threads is usually less clear than in the pro-

phases, a condition which seems to be connected purely with the spatial relations inside the cell. Thus by reference to Fig. 11 it will be seen that in the prophases the nucleus and the cell wall are closely approximated adjacent to the centriole, so that the ends of the threads are automatically located in the same optical plane. When the nuclear membrane breaks down, however, the spindle region is relatively small in comparison, and the mitochondria tend to spread out in the extra space, though they never encroach on the spindle area proper. So it happens that the striking effect of polarization is somewhat obscured, but it is perfectly clear that the orientation remains fundamentally unaltered.

The chromosomes now divide and the daughter plates begin to draw apart (Fig. 16), while the cell itself elongates very considerably, so that the distance between the centrioles is increased. The result of these movements upon the mitochondria is twofold. In the first place the polarized ends of the threads are straightened out along the spindle and in the second place they are drawn along with the diverging centrioles. As an obvious result the opposite ends of the threads are passively drawn out of the equatorial tangle, and in an early anaphase (Fig. 16) the threads, now arranged in lines more or less parallel to the spindle, come to form a sort of "palisade," or sheath, encircling the mitotic figure. The constriction of the cell wall meanwhile develops rapidly and soon comes in contact with the mitochondrial palisade. The threads, however, show no tendency to divide autonomously or to be divided mechanically, and as the constriction deepens they are carried inward by the advancing furrow. Thus the palisade as a whole is constricted very markedly in the equatorial plane, while the opposite ends flare widely, leaving an open space in which lie the daughter chromosome groups. Finally, the constriction reaches the spindle, which, by its tendency to persist as a connection between the daughter secondary spermatocytes, appears to be of a rather firm consistency. The mitochondrial threads which happen to lie immediately in the path of the constriction are thus at last caught between the spindle and the cell wall and seem to be severed mechanically into two parts (Fig. 17). That the threads are actually cut in two, not merely withdrawn into the daughter cells, is made practically certain by the whole series

of anaphase phenomena, and this interpretation is strengthened by a study of late anaphases in which a few threads still cross from one cell to the other while the majority are already severed (Fig. 17).

The arrangement of the threads with reference to the spindle is soon obscured and they spread out in each second spermatocyte in preparation for the next maturation division which occurs immediately. As many cytologists have described (see the accounts of Wilson ('12) and Montgomery ('11)), during the anaphases of the first maturation division the halves of each centriole separate in a direction at right angles to the old spindle, so that the main axis of the second maturation spindle is at right angles to that of the first. The centrioles are indeed already in position at the conclusion of the anaphase, so that the distribution of the threads in the daughter cells can be followed with reference to a possible further influence by the centers. As a matter of fact, the threads are rearranged around the centrioles as before, and the second maturation division is in general a half-size replica of the first, at least up to the cutting in two of the "palisade." The formation of the nebenkern from the mitochondria thus distributed to the spermatid will be taken up in the next section.

In the large cell generations the facts are essentially the same, but the mitochondria are comparatively short, heavy rods with numerous granules intermixed. These rods are oriented around the centrioles in a most striking manner. Their behavior at the close of the first maturation division is of special interest, for at this time the influence of the centrioles on the rods is very clearly demonstrated. In fact, as the "palisade" breaks up the rods can be seen diverging toward the centrioles in two groups, showing with the greatest clearness that the movements of the mitochondria during the entire division period are very definitely oriented with respect to the centrioles.

To summarize the behavior of the mitochondria in preparation for the formation of the sperm, it may therefore be said: (1) that in the early spermatocytes the mitochondria form a dense, nuclear "cap," (2) which is broken up during the growth period and distributed in a tangle of threads throughout the cell; (3)

that in preparation for and during the maturation divisions the threads are under the visible "control" of the centrioles, (4) and that by this means each spermatid receives an accurate quarter of the mitochondria contained in the first spermatocyte.

One important cytoplasmic structure remains to be considered, viz., the *Golgi apparatus,* a cell element whose rôle in spermatogenesis has thus far been largely mistaken or overlooked, but which, as I hope to show, must play a significant part in all future analysis of the sperm. The term "apparatus," in its original meaning, is rather a misnomer in the Hemiptera, for in these forms this structure is generally composed of many separate *Golgi bodies* (Fig. 7). To one long accustomed to the appearance of Flemming preparations of insect germ cells, the Golgi preparations are a revelation; for the mitochondria and chromosomes fade into a shadowy background upon which the Golgi bodies appear in brilliant black. Indeed a successful preparation is so nearly diagrammatic that the main outlines can be made out almost at a glance. For the purposes of this report, I have abridged the description of the Golgi bodies, attention being centered principally on their behavior in the maturation divisions. This account also is based on *Euschistus.*

For a starting point we may, as in the case of the mitochondria, take the spermatogonia in the "rosette" stage. In these early generations of spermatogonia the Golgi apparatus consists of a few scattered bodies which show no particular orientation with respect to any other part of the cell. In later generations the number seems to be slightly increased and a tendency for the bodies to collect in the neighborhood of the mitochondrial "cap" is apparent. Finally in the last spermatogonial generation (or possibly stage *a* of Wilson), this orientation is completed (Fig. 1), and the Golgi bodies are definitely restricted to that portion of the cell in which the mitochondria are aggregated. In dividing spermatogonia the Golgi bodies are broken up into small granules which seem to have no definite method of distribution to the daughter cells.

The synaptic stages offer nothing of interest but, as the mitochondria spread out in the diplotene, the Golgi apparatus likewise enters upon a period of growth and activity. As in the case of

the mitochondria the early steps in this process are much clearer in the large cells, but in most respects the small cells are the same, and important differences will be pointed out by way of comparison. In the large cells the individual Golgi bodies spread out more or less as do the mitochondria and at the same time begin to increase in size (Fig. 4). Growth is rapid and is continued up to the end of the growth-period proper. The Golgi bodies soon begin to migrate out into the general cytoplasm, their movement seeming to be approximately synchronous with that of the mitochondria. Thus they soon come to be scattered in hap-hazard fashion throughout the cytoplasm (Fig. 6), a condition which is maintained throughout the growth-period. Their number seems to be about twenty-five or thirty but it is not possible to make a very accurate count because each cell extends over several sections and the bodies may vary as to impregnation. The point particularly to be emphasized is that the Golgi bodies never at any time form a condensed aggregate in any way comparable to the so-called "Nebenkern" of pulmonates or the idiosome or "sphere" of many other forms—particularly vertebrates.

In the small cells a similar growth and migration of the Golgi bodies occurs, but it is characteristic of these generations that the general distribution is preceded by a stage in which the Golgi bodies become more or less completely condensed into one or several masses. Fig. 2 shows a cell in the early growth period in which most of the Golgi elements have condensed into a single body reminiscent of the idiosome of the opossum as figured by Duesberg ('20). It should be noticed that even in the case figured, a few Golgi bodies have failed of incorporation in the "idiosome" and usually the fusion is much less complete, resulting in several masses with numerous outlying, separate bodies. The condition in the large cells (where this phenomenon never occurs), together with the appearances often found in material fixed with Flemming and the customary failure to form a close aggregate even in Golgi preparations, leads me to believe that the fusion may possibly be in part a result of poor fixation. However, the constancy of this phenomenon argues something in favor of its reality, and the similarity to the idiosome and "Nebenkern" is very suggestive. It is this body which Montgomery

called the "sphere" (see Montgomery ('11), Fig. 48). His figures are characteristic of preparations fixed for instance in Benda's Flemming, in which the shape of the "sphere" seems certainly to be an artifact.

The shape of the Golgi bodies is much the same in all the cells, but their size varies with that of the cell as a whole. In the early growth stages (Fig. 4) the bodies appear as rods, straight or curved, which impregnate intensely, and when they have become large enough to admit of a closer study of their structure it becomes evident that the intensely black portion is accompanied by a substance which impregnates little or not at all (Fig. 6). Usually the rods are bent around this substance enclosing it more or less completely. The two portions taken together form the Golgi bodies, which are *plate-like* and not spherical. Viewed on edge, these plates or discs present a most characteristic appearance, for the rods are actually double and thus the impression is
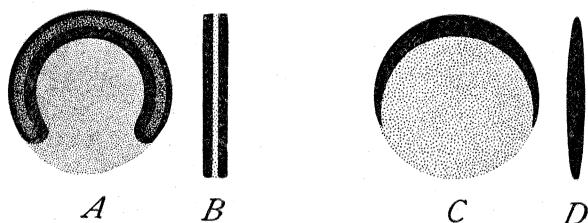
TEXT-FIGURE 1. Schematic representation of a single Golgi body. *A*, after fixation by the "modified Kopsch" method; *B*, the same as seen on edge; *C*, after fixation and staining by chrome-osmium-hematoxylin methods; *D*, the same as seen on edge.

given of a pair of rods lying side by side (Text-figure 1*b*) and separated by a clear space. So constant is this apparent "split" that from very early stages until the final condensation of the Golgi apparatus in the spermatid, it is the most striking characteristic of this kind of technique. In the late growth period in *Euschistus,* the structure of the Golgi bodies is especially clear (Fig. 7). Each disc-like body is clearly composed of two substances, a peripheral portion impregnating very densely and enclosing, sometimes completely, a plate of material which is only slightly blackened. The peripheral portion often appears as a double rim, the meaning of which is not clear (Text-figure 1*a*).

Viewed on edge the bodies exhibit the characteristic "split" (Text-figure 1b)—a feature very possibly of wide occurrence as it seems to be referred to in several published accounts of the Golgi apparatus. Following the so-called chrome-osmium methods much used by Gatenby, the appearance is quite different (Text-figure 1c). The peripheral rim stains only as a dark crescent on one side of the disc of non-staining substance ("archoplasm" of Gatenby)—or occasionally the rim may stain more completely, approaching the result obtained by impregnation with osmic acid. By these methods I have, however, been unable to demonstrate the "split" in an edgewise view (Text-figure 1d). Without going further into the morphology of the Golgi bodies, I would like especially to call attention to their obviously duplex structure as demonstrated by the staining reactions, a feature to which I shall return in a later section.

In the final growth period of the large cells it is not uncommon for the Golgi apparatus to undergo secondary changes. Thus in *Euschistus* the bodies tend to fuse with each other, the number in a single cell being reduced to ten or twelve. In *Brochymena* this fusion is carried much further, and great, amorphous masses are formed, while in *Nezara,* on the other hand, the individual bodies are very small. That these various morphologic differences are of no particular importance is indicated by the fact that in the subsequent divisions the general behavior is the same in all three genera.

The spermatocytes now enter upon the prophases, and until the spermatids are finally formed the behavior of the Golgi bodies is of great interest. The coincident series of events through which the mitochondria and the chromosomes are passing should be borne continually in mind, for only by such a composite picture can one form a proper conception of the mitotic process as a whole. As in case of the mitochondria, it is the small cell generations which offer the best material for the general study of the Golgi bodies during the prophase and division stages, and I will accordingly refer to the large cells only by way of comparison.

The early prophase probably marks the beginning of the steps preparatory to the division of the Golgi apparatus, but it is only

in the middle prophases that the results become clearly evident. Then it appears that the Golgi bodies, moving from all parts of the cell, have been gradually forming a narrow belt or girdle encircling the nucleus in what subsequently proves to be the equatorial region. Viewed from one pole, the discs form a more or less complete ring (Fig. 8) and are in general so oriented that their flat surfaces are turned toward the nuclear membrane, the edges with the characteristic " split " being thus directed toward the observer. The middle prophase now passes rapidly into the late prophase and in this stage the ring of Golgi bodies undergoes a most characteristic change. This consists of an autonomous fragmentation of all the Golgi bodies so that in the place of the ring of large discs, there is now a throng of much smaller bodies (Figs. 9 and 10). In some, a composition similar to that of the growth period discs can often be made out, but usually from this point on the morphological distinctions become more or less obscure owing to the small size of the bodies and faults in impregnation. In the small cells each Golgi body appears to be divided into two parts, but I do not wish to emphasize this point. In the large cells each of the Golgi elements fragments into many pieces, and the process of fragmentation can actually be followed. It is certain that the division is always at right angles to the " split," and the latter, therefore, however well adapted it may appear for purposes of division, never serves that end. Further, in the large cells the division clearly includes the central portion of the disc, resembling very strongly the figures given by Gatenby as representing the division of a Golgi body (see Gatenby ('19b), Fig. 14). The nuclear membrane now fades away and the metaphase figure is rapidly formed.

Before taking up the actual division stages, a word may be said concerning the nomenclature of the Golgi elements. The scattered condition of the Golgi apparatus throughout the growth period renders the use of the term Golgi " bodies " a most convenient one. Their fragmentation introduces a complication which for practical purposes I propose to avoid by the use of the term *dictyosome,* a word coined by Perroncito (see especially his paper of 1910) to denote the pieces into which the Golgi apparatus was divided during mitosis. If its meaning be re-

stricted in this original sense, the term may prove very useful, especially in such a case as the present one.

To return to the metaphase figure, apparently by the time the spindle is completed the ring of dictyosomes has already begun to spread out, and soon these bodies migrate throughout the space between the centrioles and peripheral to the spindle. At first all semblance of order is lost and the dictyosomes seem to be scattered at random around the spindle. But presently it can be seen that they are migrating toward the poles of the spindle in a very orderly way, and as the equatorial region gradually becomes clear, they are seen to be collecting in approximately equal groups in the vicinity of the centrioles (Fig. 15). In *Euschistus* this grouping is always somewhat loose, especially in the large cells, but in *Brochymena* (Fig. 15) the dictyosomes collect in two very accurate rings encircling the poles of the spindle, and presenting in polar view a most characteristic appearance.

During this entire process of *dictyokinesis*[1] the chromosomes retain their position in the metaphase plate, and only when the two groups of dictyosomes have completed their journey toward the centrioles do the chromosomes resume their activity as the anaphase begins. As the cell draws out, the groups of dictyosomes are also separated, so that for a brief interval the separating chromosome plates lie in a clear intermediate zone. Soon, however, they overtake and pass through the ring-like groups of dictyosomes and, as the daughter chromosomes complete their movement to the spindle poles, the groups of dictyosomes break up and begin to wander out through the general cytoplasm of the cell, becoming scattered apparently in all directions. During the period just prior to, and following the final anaphase the dictyosomes seem to undergo a second fragmentation, so that those of the second spermatocytes are markedly smaller in general than were those of the first. The dictyosomes form a belt, rather less definite in contour, around the chromosome plate of the second maturation division, and the process of dictyokinesis is repeated. Indeed, the steps are so similar to those of the first maturation

[1] A term employed by Perroncito to denote the division phenomena of the Golgi apparatus. Compare the parallel term, *karyokinesis*. See especially, Perroncito ('10).

division that without some familiarity with the particular genus one happens to be observing it is impossible to say off-hand whether the division is that of a first or second spermatocyte. The fate of the dictyosomes in the telophase of the second maturation division will be taken up in connection with the formation of the sperm.

To summarize the behavior of the Golgi apparatus in these stages: (1) in the early spermatocytes the Golgi apparatus consists of a number of scattered bodies which in later spermatogonial generations are collected around (and in?) the mitochondrial cap; (2) during the growth-period these bodies spread throughout the cell, and take part in its general growth; (3) they are finally accurately oriented with respect to the first maturation-spindle apparently through the influence of the centrioles; (4) then, having undergone autonomous fragmentation, the resulting dictyosomes are actively distributed during the maturation division under the visible control of the centrioles, (5) by which means each spermatid receives an accurate quarter of the Golgi apparatus present in the first spermatocyte.

III. *The Formation of the Sperm.*

So intricate and extended are the processes by which the mature sperm is differentiated from a spermatid, that at this time I can scarcely do more than indicate the main features, emphasizing a few points of special interest. The great variety of technical methods employed have brought to light many new features in the spermatogenesis of these Hemiptera, a few of which may be mentioned, but in the main they must be left for treatment in a later paper.

The general topography of spermiogenesis has been worked out on *Murgantia,* chiefly because the small size of the cells offers many advantages in studying the later stages, where at best the sperm heads are inconveniently long. The transformation of the spermatid nucleus into the sperm head has been described for *Euschistus* by Montgomery ('11) who carried his study to a stage which he called a mature sperm, describing the head " as a thin hollow cylinder of chromatin, containing a distinct cavity filled with nuclear sap." From his figures and his statement that

"no evidence was found for the casting off of any substance by the sperm," it is abundantly clear that Montgomery failed to observe the final steps in the differentiation of the sperm head. In a stage following shortly on the last one described by Montgomery, the head undergoes a characteristic change resulting in what appears to be a complete vacuolization of the chromatic lining. Then the chromatin collapses toward the axis of the head, and a thin, compact thread is formed running throughout the length of the head and enclosed in a protoplasmic envelope which appears to correspond to the outline of the old head (Fig. 33). So far as I know, this stage in the transformation of the Hemipteran sperm has not been noted heretofore, but the description given by Faust ('13) of the mature sperm in *Anasa tristis* corresponds very closely to the stage just described in *Murgantia*. Possibly this phenomenon may prove to be much more generally distributed among the Hemiptera than has been suspected. That this is not a mature sperm is quite clear in my material, for subsequently the outer protoplasmic envelope disappears and the head of the completed spermatozoön is merely a rod of chromatin (Fig. 34) corresponding to the descriptions of many authors.

Such in brief is the course of events in the small cell generations of spermatids, but in the large cells it is quite different. In these, the layer of chromatin lining the nuclear membrane, so characteristic of the small spermatid, is from the first more or less vacuolated, and soon after the head begins to elongate (see Montgomery ('11), Fig. 141) the layer breaks down completely. The head now rapidly elongates, greatly exceeding the small sperm in this particular, while the chromatin content remains in an indefinitely vacuolated condition. Finally, the chromatin condenses into a more or less completely spiral thread which ultimately forms an axial rod as in the case of the small sperm, but of very much greater length (Fig. 35).

In brief, the two sizes of spermatids in *Murgantia* yield sperms of very different lengths by processes of nuclear differentiation which, while superficially unlike, prove to be fundamentally similar.

The centrioles in the Hemipteran spermatid have been so

variously described, that a comparative analysis is out of the question in this paper. However, in view of the fact that I have been able to clear up some of the doubtful points in Montgomery's ('11) description of *Euschistus*, a very brief statement of the facts may be made as I have found them in *Murgantia*. In the very young spermatids the centriole is not single as Montgomery stated, but distinctly double (Fig. 22). This is especially clear at the time when the centrioles are migrating from their original anterior position to their definitive position between the nebenkern and the nuclear membrane. At this time they are always placed in a line normal to the nuclear wall, so that the proximal centriole touches the nucleus while the distal one gives rise to the axial filament of the tail (Fig. 22). Arrived in their position at the future base of the head, they undergo a change in orientation and for a short time their duplicity is lost, and only a single granule-like centriole can be distinguished. A little later, when the halves of the nebenkern have started to elongate, the two centrioles can again be made out in favorable cases. They appear in the form of two short rods, joined at one end to form a "V," at the vertex of which the axial filament is inserted (Fig. 26). At first the rods lie tangentially (or nearly so) to the nuclear membrane, and as seen from the side give the impression of a large granule to one edge of which the axial filament is attached, an asymmetry noted by Montgomery. Then the stray chromatin masses which have not taken part in the formation of the chromatin layer on the inner surface of the nuclear membrane begin to collect in the vicinity of the centrioles where they eventually form a compact and conspicuously staining body (Fig. 29). This body I have called the *pseudo-blepharoplast* because it has always been regarded as the centriole of the spermatozoön. Indeed the pseudo-blepharoplast so easily obscures the true centrioles, that lacking a study of earlier stages its real nature would certainly be overlooked. The rod-like centrioles eventually straighten out parallel to the long axis of the sperm (Fig. 29), and somewhat later the pseudo-blepharoplast fades away, as though it were dissolved in the nuclear sap. This phenomenon was correctly described by Montgomery who was naturally puzzled by the apparent solution of the spermatid centriole

in the nuclear sap. The apparent anomalies of the Hemipteran centrioles are thus cleared up, and they are seen to conform with the usual types of sperm formation.

To summarize: (1) in the spermatid the customary pair of centrioles is present, which (2) remain in the " neck " region and give rise to the axial filament of the tail; (3) the centrioles in early stages are accompanied by a chromatic pseudo-blepharo-plast, which is subsequently resorbed by the nuclear sap.

The fate of the spermatid mitochondria in *Euschistus* has been outlined by Montgomery ('11) and the much more complete account which I have worked out is not essential for the present purpose. Suffice it to say that the threads allotted to the spermatids by the second maturation division are rapidly condensed by characteristic steps into a compact sphere (Fig. 22) (the spermatid *nebenkern* of authors), which soon divides into two equal parts, and these, rapidly elongating, come to form a sort of mitochondrial sheath for the axial filament which lies between them. During the intermediate stages in the elongation of the nebenkern halves they develop a series of paired bleb-like swellings (Fig. 27) which increase in number as the mitochondrial sheaths spin out (Fig. 32) and are the most conspicuous thing in Benda preparations of this period. Subsequently they seem to be reabsorbed and the sheaths become mere threads running very nearly the entire length of the tail of the sperm. These vesicular swellings on the sheaths are to be found in a number of insects, as I have ascertained, but I find scarcely any direct mention of them in acounts of spermatogenesis; except in Duesberg's ('10) work on *Blatta* where something of a similar nature occurs. In the mature sperm the two sheaths appear to retain their thread-like structure showing no tendency to fuse into a complete mantle for the axial filament as is the case in *Blatta* according to Duesberg. Smears of mature sperm show clearly that the tail is flat and ribbon-like, each edge being formed of a distinct thread—the derivative in all probability of one of the original halves of the spermatid nebenkern.

I have, further, made some interesting observations on the structure of the mitochondria as revealed especially in the divided halves of the nebenkern during the early stages of their elonga-

tion. Students of the mitochondria in Mollusca and Lepidoptera have long supposed the chondriosomes to be composed of two different substances—an outer chromophilic envelope staining intensely with crystal violet and related stains, and an inner, medullary or "chromophobe" material which stains little or not at all with all the usual reagents. By means of Cajal's Golgi method I have succeeded (Bowen, '19) in impregnating this medullary substance with the greatest clearness, the chromophilic envelope being left quite colorless and transparent. Fuller treatment of these observations lies beyond the scope of this paper, but I would like to emphasize that a duplex chemical structure has thus been conclusively demonstrated at least for some mitochondria.

Finally, it remains to trace the rôle of the Golgi apparatus in the differentiation of the sperm—a subject to which a rather more complete treatment may be accorded by reason of its great theoretical importance. The preceding section gave an account of the Golgi apparatus up to the final anaphase of the second maturation division, when the daughter chromosome plates have completed their journey to the spindle poles and the dictyosomes are grouped in two clusters close to the chromosome plates and encircling the spindle remains (Fig. 18). I have followed out the further stages in both Brochymena and Euschistus, where they are essentially the same. In Brochymena the mitochondria seem to condense directly into the nebenkern, while the dictyosomes, already fusing with each other to form larger masses or Golgi bodies, are scattered over its whole available surface (Fig. 19). They are always thus closely in contact with the nebenkern, showing no tendency toward a more general distribution, and until the final fusion of all the Golgi elements they retain this close connection with the surface of the nebenkern. In Euschistus, on the contrary, the Golgi bodies while remaining in the vicinity of the nebenkern tend to be rather scattered.

The fusion of the Golgi bodies continues rapidly, their number constantly decreasing while their size correspondingly increases (Fig. 20). Soon definite little aggregates more or less plate-like or spherical in shape are formed whose periphery impregnates heavily just as did the Golgi bodies in the growth period, this

heavily impregnated layer being characteristically absent on the side in contact with the nebenkern. In a short time the fusion has progressed so far that only three or four separate elements can be distinguished, and these too, continuing the process of fusion, soon unite to form a single mass of rather regular shape and dimensions (Fig. 21), which constitutes the "sphere" or idiosome of many writers on spermatogenesis. In the final stages of fusion the Golgi elements have tended to collect in the groove between the nuclear membrane and the nebenkern (Fig. 20), so that when its formation is completed, the single, condensed mass is always similarly situated (Fig. 21).

The dictyosomes distributed to the spermatids during the maturation divisions thus fuse to form a real Golgi apparatus, from which the acrosome is to be derived; and I therefore propose to call it the *acroblast*.[1] This term was first suggested by King ('07) and was subsequently adopted by Gatenby ('17) for designating in cells of various stages certain bodies supposed to give rise to the acrosome. The nature of these bodies in Miss King's work is doubtful, but in the case of Gatenby the acroblasts are certainly the Golgi bodies. It seems to me a procedure of little practical use and of very doubtful theoretical value to extend the term "acroblast" to structures present in the cell prior to the actual formation of the spermatid. In the first place, as I shall try to bring out later, it is highly probable that the Golgi apparatus is always the source of the acrosome, and it is clearly not desirable to use "acroblast" merely as a synonym for Golgi body or Golgi apparatus. Furthermore, cytologists are in actual want for some purely descriptive term which can be universally applied to the anlage of the acrosome without necessarily involving any implications as to origin and homology such as have so beclouded the terms "idiosome" and "sphere." I therefore propose to restrict the term *acroblast* to that body or bodies[2] from which the acrosome is actually derived.

[1] My attention was first called to this very useful term by Professor Wilson.

[2] I use the word "bodies" because there are probably cases in which the acrosome is originally multiple, the parts later fusing into a single body, as for example in Lepidoptera, according to Gatenby ('17). In fact this occasionally happens in the Hemiptera, indicating that the small aggregates which finally fuse to form the definitive acroblast are each potentially acroblasts in miniature.

The acroblast is a more or less hemispherical body, pressed close to the nuclear membrane (Fig. 21), the periphery of which impregnates very heavily except for the portion touching the nucleus, where the substance which takes the osmic acid seems to be absent. The substance within the peripheral layer blackens very little and the picture as a whole recalls the similar arrangement of substances in the Golgi bodies of the growth period. The acroblast is completed at about the time the centrioles migrate around the nucleus to their position near the nebenkern, and is always located on the side of the nebenkern opposite that on which the centrioles will come to lie. As the centrioles migrate into their definitive position, the acroblast moves around the opposite side of the nucleus to an anterior position (Fig. 23). After pausing for a time (Fig. 24) it continues on around the nucleus, retracing the path over which the centrioles had traveled, and eventually comes to rest close to the centrioles (Fig. 25) but, with reference to its former position, on the opposite side of the nebenkern. Here it remains until the final step in the formation of the acrosome is completed.

As the acroblast slowly makes its way around the nucleus of the spermatid it undergoes a process of differentiation which, there is reason to believe, is of fundamental importance. The first evidence of this is the appearance at the base of the acroblast—that is at the surface next the nuclear membrane—of a transparent, spherical vesicle or bubble (sometimes there is more than one) which seems to be forming out of the colorless substance inside the acroblast (Fig. 23). At first it is very small but, growing rapidly, it gradually protrudes farther beyond the border of the acroblast which is thus lifted away from the nuclear membrane (Fig. 24). This transparent, structureless vesicle is to be the acrosome, and by this name it may be conveniently referred to even at this early stage in its formation. It is always in contact with the nuclear membrane while the acroblast fits over it like a shell. As the acrosome continues to enlarge, the acroblast seems to diminish somewhat in size, apparently at the expense of the non-staining substance, and eventually comes to form a mere appendage to the acrosome itself (Fig. 28). At the point where the acrosome touches the nucleus, a small granule

is characteristically developed within the clear vesicle (Figs. 25 and 27), but this is not visible as a rule in Golgi preparations.

The spermatid is now ready to proceed with the general processes of differentiation. The acrosome shrinks somewhat, appearing to express some of its contents into the acroblast, and begins to move around the nucleus toward the anterior end (Fig. 30). As it does so its whole appearance changes; it becomes more opaque and begins to stain darkly with Fe-hematoxylin. Simultaneously the acroblast begins to draw away toward the tail region, and soon the connection with the remaining portion is severed (Fig. 31). The latter continues its journey around the nucleus, and becomes the acrosome while the former is carried back along the tail. The term *acroblast* is scarcely applicable to this cast off remnant (Fig. 31), nor is it any longer to be identified with certainty as Golgi apparatus, and I shall therefore refer to it merely as the " *Golgi remnant.*"[1]

For a while the Golgi remnant can be followed as it moves back along the tail, but it soon becomes lost in a group of granules from which it can not be certainly distinguished, and which are doubtless eventually cast out of the sperm with the protoplasmic ball sloughed off from the tail. It is practically certain that the Golgi remnant is cast off along with the other débris— at least I have never been able to find any traces of it in the last stage. Montgomery's ('11) statement that no protoplasm is eliminated from the sperm is certainly mistaken, for I have followed every step in the casting off of the protoplasmic balls and have ascertained that they are eventually ingested by the epithelial (nurse) cells which line the cavity of each cyst.

CRITICAL CONSIDERATIONS.

I. *Problems of Cytoplasmic Division.*

As yet our knowledge of exact processes in the distribution of the formed elements of the cytoplasm in cell division is exceedingly limited and we are, indeed, accustomed to call attention to

[1] This cast off body is homologous with the *idiophthartosome* of Papanicolaou and Stockard ('18) and the *idiozomrest,* etc., of many authors. We are really much in need of a convenient and purely descriptive term for this body.

the contrast between the accurate, meristic division of the nucleus and the supposedly haphazard, mass division of the cytoplasm as one of the striking features of karyokinesis. In part, no doubt, we have been led to an easy acceptance of this view by what appear to be the necessary implications of the modern chromosomal theory of heredity, which, by its emphasis on the exactness of chromosome distribution, perhaps predisposes us to assign a secondary importance to the division of the specific components of the cytoplasm. Such a standpoint is unwarranted, for while it may be improbable that any mechanism will ever be made out for an exact division of the cytoplasm comparable to that of the nucleus, it does not necessarily follow that cytoplasmic division can be reduced to the basis of a mere mass division subject only to the laws of chance. Nevertheless, at the present time cytoplasmic division seems often to be treated as if it were a haphazard process, the essential phenomena of which are the same whether cell division be mitotic or amitotic, with of course an implied exception in the case of the centrioles and the achromatic division figure.

It seems to me, however, that the facts observed in the Hemiptera point rather to a conception of cytoplasmic division as a precisely ordered process. That the cytoplasm may indeed undergo division of a remarkably regular character has long been recognized in the special case of the segmenting egg; and in the so-called "determinate" eggs particularly of molluscs and ascidians the experimental studies of recent years have revealed a very definite order in the distribution of cytoplasmic factors of differentiation which presumably are traceable to corresponding "formative stuffs." For accomplishing such complicated cytoplasmic divisions some special mechanism seems necessary, but as to its nature very little has been made out. In the division of tissue cells, however, appeal can not be made, as in the case of the egg, to direct experiment, and we must here rely on morphological studies alone. An approach to the problem may therefore be made by a comparison of the quantitative relations existing between the products. Accordingly the problem of cytoplasmic division may be stated for present purposes in the form of a double question: (1) Is the division of the cytoplasm

and its formed elements strictly regular as regards the quanti-
ties involved? (2) If so, what is the mechanism for achieving
such a result?

For an answer to these questions we can have recourse to no
more suitable material than is offered by the two spermatocyte
divisions. In these cases, if anywhere, we should expect to
find conclusive evidence of an exact regularity in division, for
the spermatocyte divisions and the subsequent differentiation of
the sperm are accomplished so rapidly that there is little time for
the correction of inequalities in division by differential growth.

As a matter of fact the mass distribution of the cytoplasmic
elements has been studied by many workers with very uniform
results. Attention has thus far been centered chiefly on the mito-
chondria, and Duesberg ('10) has well summarized this aspect
of the question, showing conclusively that the spermatids arising
from a given spermatocyte contain each a fourth part of its orig-
inal mitochondrial content. This general conclusion is corrobo-
rated with the greatest clearness by the case of the scorpion *Cen-
trurus* described by Wilson ('16) in which each spermatid re-
ceives accurately equal portions of a remarkable chondriosome-
ring characteristic of the primary spermatocytes. To be sure,
in another scorpion, *Opisthacanthus,* the distribution is some-
times demonstrably uneven, but the discrepancies are always
slight. My own observations on the Hemiptera show that the
mitochondria are rather accurately halved at each maturation
division, with the result that each spermatid receives an approxi-
mately equal mass of chondriosome material. On the other hand
there are cases in which the distribution is markedly unequal,
especially in forms in which the spermatocyte divisions are ab-
normal, as for example the honey-bee (Meves, '07). Of even
greater interest are the inequalities in the mass distribution of
the mitochondria in the segmenting eggs of the ascidian (Dues-
berg, '15). It is clear, therefore, that the division of the mito-
chondria considered merely from the standpoint of the quantities
involved is very often equal, but in certain special cases quite the
contrary. In any particular case, however, the distribution ap-
pears to be always approximately the same. In other words,
there is a definite *regularity* in the amount of mitochondria dis-
tributed to a given cell.

In the case of the Golgi apparatus, the evidence is as yet very meager, but none the less positive. Gatenby ('18) has recently given some account of dictyokinesis in several genera of pulmonates, and in the *Limacidæ* at least he found evidence of a very exact distribution of the Golgi elements, corroborating the similar observations of Platner ('89) made many years ago. Although his observations were not made on the germ cells, Deineka ('12) has given one of the clearest accounts of dictyokinesis so far published, and he points out the apparently equal distribution of the dictyosomes to the daughter cells. The observations on Hemiptera which I have already given furnish, it seems to me, the best ground on which to base an opinion; for the essentials of dictyokinesis are so clear that it is possible to arrive at a reliable conclusion. And indeed it may be affirmed that the Golgi apparatus of the spermatocytes is divided very equally among the spermatids, so that each receives a fourth of the original amount. Accordingly, in the case of the Golgi apparatus there is also a definite regularity in the quantities distributed in division to the daughter cells.

Concerning the mass of cytoplasm proper, an approximately equal division often occurs, at other times a very unequal one, but in any case the same regularity is a characteristic feature. Summing up the evidence, therefore, our first question concerning the existence of regularity in the mass division of the cytoplasm may be answered affirmatively. There *is* conclusive evidence that the cytoplasm and its principal formed elements are distributed with great regularity by the processes of cell division, the most usual result being that each daughter cell receives a rather accurate half of the total quantity of cytoplasmic materials.

Concerning the second question, namely, as to the mechanism by which this regularity in mass distribution is achieved, two answers at once suggest themselves: (1) The division may be a purely chance separation of elements distributed at random in the cytoplasm; or (2) It may be accomplished by definite activities related to those which produce the spindle and the resultant phenomena of karyokinesis. Thus far the relation of the cytoplasmic elements to the centrioles and the spindle in general has received scant attention, and the idea of chance distribution has

been rather tacitly accepted as an explanation. My observations on *Euschistus* do not bear out this view, and I should like therefore to make a brief survey of the problem as a whole in an effort to find, if possible, a consistent basis for the whole series of observations.

It has often been pointed out that the chondriosome-material in dividing cells (spermatocytes, and others) tends to appear in the form either of granules or of rods and threads of various dimensions. In fact one can arrange these various morphological types in a rather instructive series. There is first the case in which the chondriosomes are scattered rather evenly throughout the cytoplasm in the form of small granules, as, for example, in the guinea pig (Duesberg, '10). An orderly arrangement with respect to the achromatic division figure here seems entirely lacking. In many pulmonates (Gatenby, '18) conditions are very similar except that the granules are much larger, and in many Lepidoptera (Gatenby, '17) enlarge to form vesicular bodies, in some cases few in number and of considerable size. From such a condition it is a short step to that in the scorpion *Opisthacanthus*, where a definite(?) number of " chondriosome spheres " occur, involving a further element of regularity which seems lacking in the other cases mentioned. All the chondriosome formations so far considered agree in one particular; they do not appear to be subject to any influence other than that of chance. Only in a single case, so far as I know, has a definite orientation of small scattered chondriosomes been described. This is the condition in *Ascaris* described by Hirschler ('13) where the slightly elongate chondriosomes are all distinctly oriented toward the centrioles. This observation should be confirmed, for if correct it throws much light on the whole mechanism by which granular chondriosomes are distributed in cell division.

The chondriosome-ring of *Centrurus* offers an interesting introduction to the rod type of mitochondria, for after becoming drawn out along the spindle it breaks apart into two rod-like halves which are subsequently divided across and the parts distributed to the daughter cells. It is only a step from this case to that of *Paludina* (Gatenby, '19a) where there is a limited number of heavy rods which become arranged along the spindle in

the telophase and as in the ring derivatives of *Centrurus* are sub-
sequently divided. In these two cases there appears for the first
time a relationship between the spindle and the chondriosomes
which is essential to an equal, mass division. In *Blaps* (Dues-
berg, '10) the mitochondrial rods are very numerous and much
smaller in diameter, and, what is of most interest, are oriented
toward one pole of the cell during the prophases of the first
maturation division. What relation this bears to the centrioles
is not stated, but the arrangement is clearly a forerunner of the
"palisade" of rods which encloses the spindle during division,
and is divided across as in the two preceding cases. A further
step in the definite orientation of the mitochondria with respect
to the spindle is found in the case of the honey-bee described by
Meves ('07). In the bee the early history of the mitochondria is
very similar to that which I have described in *Euschistus,* the late
growth period being characterized by a tangled mass of threads
enveloping the nucleus on all sides. During the prophases, how-
ever, the threads are all aggregated into a single tangled mass
situated opposite that centriole which ultimately comes to lie in
the functional sperm, and therefore presumably in the vicinity
of the other centriole. As the spindle forms, free ends of threads
begin to appear around the edge of the mass of mitochondria ;
and these gradually move toward the opposite centriole until the
whole mass is untangled and the threads stretch from centriole
to centriole, parallel to the spindle and enclosing it more or less
completely in a mantle of threads. Wilke ('13) has also de-
scribed the orientation of mitochondria toward the centrioles in
the spermatocyte divisions of *Hydrometra,* but his figures give a
rather inadequate idea of the exact chain of events. Finally, the
case of *Euschistus,* described in an earlier section, demonstrates
conclusively that the arrangement of the mitochondria prepara-
tory to division depends in these forms upon some influence that
is as it were focused in the centrioles. There is thus a complete
series ranging from a mass of scattered granules dependent for
equal division apparently on the exigencies of chance, up to a
mass of threads definitely oriented towards the centrioles to the
end that an equal division of their substance may occur. How
can these apparent contradictions be harmonized? A possible

answer to this question may be postponed until the mechanism of dictyokinesis has also been considered.

Although the Golgi apparatus has been known for over twenty years there is still scarcely a single adequate account of its exact behavior in cell division. Duesberg's ('20) recent report of the Golgi apparatus in the opossum and the account by Papanicolaou and Stockard ('18) of the guinea pig, leave much to be desired in the way of details, and it is difficult to draw any very trustworthy conclusions. It appears, however, that in these forms the Golgi apparatus undergoes a simple fragmentation, the resultant pieces being scattered throughout the cell and subsequently divided in a way reminiscent of the chondriosome granules, for example in the guinea pig. A similar distribution of previously separate Golgi bodies has been described in *Ascaris* by Hirschler ('13). Practically all other accounts agree in indicating a much more complicated process. Platner ('89) seems to have been the first to study the division of the Golgi apparatus in detail. He worked on the pulmonate *Limax*—material which has since been extensively employed for the same purpose since its Golgi apparatus seems especially resistant to acetic acid. Platner observed the breaking up of the " Nebenkern " just prior to division and the separation of the pieces into two groups which, accompanying the centrioles, migrate to opposite sides of the nucleus and when the spindle is formed become disposed more or less radially around the centriole. At the second spermatocyte division, the same process is repeated. Platner also described some interesting division phenomena of the individual rods (dictyosomes?), which have not however been confirmed in detail by later workers. The essential features to be noted in this description are the fragmentation of the parts of the Golgi apparatus and their distribution to the daughter cells seemingly under the direct control of the centrioles. Since Platner's time many others have examined the so-called " Nebenkern " of pulmonates with most contradictory results, and it must be confessed that none succeeded as well as did Platner in gaining a complete conception of dictyokinesis. Of later workers, Murray ('98) should be especially mentioned, for his observations on *Helix* and *Arion* contain many details relative to the structure of

the dictyosomes which offer most interesting comparisons with my own observations on the Hemiptera. He failed to confirm Platner's description of a very accurate splitting of each dictyosome during the division stages, but his work bears out the conclusion that some sort of fragmentation occurs like that so clearly demonstrated in *Euschistus*. Fragmentation of the original Golgi bodies or "batonettes" is also indicated by the difficulty which Gatenby ('18) experienced in staining the Golgi elements during the division stages. I have had the same experience with *Euschistus*, in which the Golgi bodies stain readily with Fehematoxylin after the proper technique but the dictyosomes fail entirely of demonstration. Gatenby ('17) also noted the aggregation of his "acroblasts" (probably dictyosomes) around the centrioles during the spermatocyte divisions of many Lepidoptera but how they attain this position was not indicated.

Deineka ('12) has described the division of the Golgi apparatus in cells of Descemet's membrane, and the general outline is distinctly similar to the type of behavior in *Euschistus*. The dictyosomes formed by fragmentation of the Golgi reticulum are arranged in an equatorial belt encircling the metaphase chromosome-plate, and thence they wander to the opposite poles of the spindle, becoming in this way divided into two distinct polar groups. Finally, in *Euschistus*, I have been able to follow the process step by step, and the relation of dictyokinesis to the general mitotic figure is completely demonstrated.

If we try now to gain some general conception of the processes by which cytoplasmic division is accomplished, we note in the first place one fundamental fact applicable to all cases. *The cytoplasmic elements are so distributed during the early division stages that the constriction of the cell wall will ultimately divide them in some regular mass proportion.* It has been held that this distribution is a matter of chance only, but I believe that the facts set forth above point rather to the existence of a definite mechanism for the regular arrangement of the cytoplasmic elements in division. Indeed, the case of *Euschistus* provides convincing evidence that the mitochondria and Golgi apparatus may undergo a division accomplished in some way by activities that focus in the centrioles. The evidence from other sources tends

to the same conclusion in respect to the division of the Golgi apparatus, except possibly in mammals where the descriptions are contradictory. With the mitochondria however the case is different, and there seems on the surface to be no general rule. May this not be, after all, only an apparent difference? It seems difficult to conceive of a purely chance distribution of mitochondria which would produce the relatively constant results in *Opisthacanthus* for example, and it is still more difficult to conceive of a chance arrangement which would allow the dividing cell to sever the chondriosome-ring of *Centrurus* into exactly equal parts. Why should chance distribute the granules in the guinea pig germ cell so evenly? Why, indeed, should the granules be distributed at all, since in earlier stages they tend to be aggregated at one pole of the cell? In this connection the observation of Meves ('14) on the dividing egg of *Ascaris* is of interest, for he found that the mitochondria in the form of very small granules tended to collect around the poles of the cleavage spindles, *i.e.*, around the centrioles. Finally there is the case of the spermatocyte divisions of *Ascaris* in which the axial dimensions of the mitochondria reveal the presence of an underlying polarization which, if the chondriosomes were spherical, would never be suspected.

If we combine these many facts, it is difficult to escape the conviction that the division of the mitochondria and the Golgi apparatus is always in more or less definite relation to the centrioles and that the regularity which has been shown to exist in cytoplasmic distribution is thus to be traced ultimately to the same influences which control the formation of the mitotic figure and the distribution of the chromosomes. I have come thus to a concept of the dividing cell as a whole which differs somewhat from that now commonly accepted. It seems to me probable that the centriole is to be considered not merely the morphological expression of the influences which construct the spindle and marshal the chromosomes for division, but that it is in a much wider sense the dynamic center of the whole cell, in which are centered at the time of division influences that extend to practically all the elements of the cell and direct their division in an orderly manner. This view of the functional significance of the

centriole takes us back again to the theories long ago proposed by Van Beneden and Boveri, which contained a nucleus of real truth that later workers have been inclined to overlook.

This conclusion leads naturally to the consideration of a further possibility, namely, that the division of the cytoplasmic structures may be actually more or less meristic in character. It is impossible in the present state of our knowledge to make any very definite statement on this point, which has thus far received little careful study. The results from the study of plant plastids indicate something, however, of the possibilities for growth, multiplication, and distribution in cell division possessed by these bodies. All that is needed to give such behavior the aspect of a meristic division is a definite mechanism for the distribution of the plastids in mitosis. That such an apparatus may exist is demonstrated clearly by the surprisingly complicated mode of division undergone by the Golgi bodies in *Euschistus,* in which a partially meristic division almost certainly occurs. Such facts as these coupled with the probable relation of many cytoplasmic elements to a mechanism for orderly division indicate a possible degree of cytoplasmic organization which has been little suspected. Unfortunately our knowledge concerning it is as yet so meager that we can scarcely even conjecture its nature and extent, but of its possibilities for a highly complex mode of division there can, I believe, be no longer any doubt.

II. *The Origin of the Acrosome.*

No structure in the spermatid has been subject to more contradictions of description and homology than the acrosome.[1] It has in general been traced to a "sphere," the relations of which to preceding cell structures have generally been merely inferred and often quite mistaken. But aside from the purely descriptive "sphere," the acrosome has been asserted to originate from the mitochondria, spindle remnants, and combinations of mitochondria and spindle remnants, from a centrosome, a centriole, an

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[1] The term "Akrosoma" was first applied by Lenhossék ('98) to the little granule within the vesicle which is produced by the "sphere" (acroblast). As a matter of fact the term has been applied so widely to the material as a whole which forms the apical body that I see little reason for attempting to restrict the word to Lenhossék's original meaning.

accessory chromosome, an acroblast, new formations in the cyto-
plasm, an idiosome of doubtful ancestry, and the Golgi apparatus.
In the vertebrates especially, where there is a very definite
" sphere " or idiosome in the spermatocytes, the similar formation
in the spermatid has been referred to the spermatocyte structures
—generally without any adequate reasons.   It is among the in-
sects, where a spermatocyte " idiosome " seems rarely to be well
developed, that the greatest confusion has prevailed.   Indeed any
attempt to extract some fundamental conception from the maze
of description has thus far seemed hopeless.   Viewing the prob-
lem as a whole, two facts alone stand out in seemingly universal
agreement, namely, (1) that there is always a body which comes
to lie at the anterior tip of the sperm and forms the acrosome of
authors, and (2) that in connection with this process there is
usually a remnant cast off and lost in the protoplasm of the tail
region.

The second of these may require some notes in explanation.
From a general survey of spermatogenesis, I have found fairly
clear evidence of the elimination of some material formerly in
connection with the developing acrosome in many cases, of which
the following are a representative selection from widely separated
groups:

Nematoda		*Ascaris* (Hirschler, '13)
Insecta	Coleoptera	*Passalus* (Schafer, '17)
		Cybister (Voinov, '03)
	Orthoptera	*Gryllotalpa* (Payne, '16)
		Locusta (Otte, '07)
	Hemiptera	*Pyrrhocoris* (Henking, '91)
		Anasa (Paulmier, '99)
		Euschistus (Montgomery, '11)
Arachnida		*Lycosa* (Boesenberg, '05)
Mollusca		*Columbella* (Schitz, '16)
		Paludina (Gatenby, '19a)
Amphibia		*Bufo* (King, '07)
Mammalia		*Mus* (Niessing, '97, v. Lenhossék, '98)
		Cavia {Benda, '91, Niessing, '97, v. Lenhossék, '98, Sjoevall, '06, and others
		Didelphys (Duesberg, '20)

Other cases can readily be found in which the casting off of a remnant by the acrosome has not been mentioned but is nevertheless indicated (see for example Terni, '14). If then the anlage of the acrosome presents this bipartite character, how is the acrosome itself derived and what is the nature of the cast off remnant? I believe that the facts which I have described in the Hemiptera offer a decisive answer to this question, and at the same time point the way to a new conception of the acrosome which may clear up the confusion in which we find ourselves at present.

In *Euschistus* we find in the primary spermatocytes a number of scattered Golgi bodies characterized by a duplex structure. On the one hand there is a heavily stained peripheral zone, the " Golgi apparatus " of authors, and on the other a central non-staining or chromophobe substance to the nature of which I will return later. In the maturation divisions these bodies undergo fragmentation into dictyosomes which, having been distributed in equal amounts to the spermatids, undergo progressive fusion until a single mass (acroblast) is formed, the " sphere " of many authors. This mass presents the same staining affinities as did the Golgi bodies of the spermatocytes, a darkly staining peripheral layer and a chromophobe interior. Although it has not been possible to offer conclusive proof of the *exact* homology of these two substances in spermatocyte and spermatid, I think it can scarcely be doubted that we have in this mass (acroblast) the direct reconstitution of the structure of the spermatocyte Golgi bodies. In other words, *the material (acroblast) from which the acrosome is to be derived is the Golgi apparatus.*[1] By a process of differentiation connected with the chromophobe material occupying the interior of the acroblast (*Golgi apparatus*), and probably at its expense, there is formed a large, vesicular body containing a small granule, which together form the acrosome of the sperm. The remnant of the Golgi apparatus is cast off and

[1] I have used the term *Golgi apparatus* as covering the Golgi bodies (and acroblast) as a structural whole, a usage which seems to be justified for the present in the light of the possibilities which are discussed in the following section on the structure of the Golgi complex. As I have there pointed out, the restriction of the term *Golgi apparatus* to the part impregnated by the customary methods is very possibly an artificial limitation.

appears to take no further part in the formation of the sperm, while the acrosome is applied to the anterior part of the sperm head and may there undergo further changes the nature of which is of no interest in this connection.

To draw a general conclusion from the facts in the pentatomids alone might well seem an unwarranted procedure; but fortunately more or less fragmentary accounts from several other animal groups are now available, and they corroborate fully the general outline which I have given. The best case is that of the opossum where Duesberg ('20) has described the origin of a vesicular acrosome with an enclosed granule from the Golgi apparatus which he proved to be directly derived from the "idiosome" of the spermatocytes. Once the acrosome is formed, the Golgi remnant is cast off. A similar though less complete account has been given by Sjoevall ('06) for the guinea pig. From Terni's ('14) figures of *Geotriton* it seems certain that much the same thing occurs in that amphibian. Further, the work of Schitz ('16) and Gatenby ('19a) clearly proves the same thing in several molluscs, though in this case the acrosome is in the form of a granule the exact homologies of which are as yet uncertain. Gatenby ('17) has also described the origin of the acrosome from Golgi bodies (his acroblasts) in the Lepidoptera, though the case is in some respects contradictory and not yet entirely clear. Lastly, Hirschler ('13) has described the partial elimination of the Golgi apparatus from the sperm of *Ascaris,* the remainder apparently forming a body which some writers have compared with the acrosome of typical nematosperms. The failure of many workers to trace the origin of the acroblast is obviously due to the difficulty of demonstrating the Golgi apparatus during cell division, and the fantastic descriptions especially among the insects are clearly the result of inadequate fixing and staining methods. From a study of a wide variety of technical methods I can testify to the great differences which are attributable to methods of preparation alone.

Drawing together these many threads of evidence, both from observation and inference, it seems to me that the following conclusion embodies a conception of the acrosome which is in accord with all the facts and which establishes its formation as an

orderly part of a logical scheme of sperm formation. The acrosome of the animal sperm arises in connection with the Golgi apparatus. When the differentiation of the acrosome has proceeded to a certain point, the remnant of the Golgi apparatus is cast off and probably plays no essential rôle in the mature sperm. The acrosome is, therefore, no fortuitous body contrived at the last minute from spindle fibers or other chance inclusions, but is to be considered in the light of a cell organ as definite in its origin and rôle as are the nucleus, centrioles and mitochondria of the spermatid.

III. *The Idiosome and the Structure of the Golgi Apparatus.*

In conclusion I would like to refer briefly to some general problems connected primarily with the structural aspects of the Golgi apparatus in the male germ cells. In the first place it is to be noted that the structures especially characteristic of the spermatocytes of pulmonates and vertebrates and appearing in the literature under the name of Golgi apparatus, idiosome, Nebenkern, sphere and other less familiar terms, are all one and the same thing in a topographical sense. They represent a complex of the Golgi apparatus plus a definite substance which forms the idiosome of Meves. Duesberg ('20) has pointed out, and with reason, that the idiosome has nothing to do with the old attraction sphere of Van Beneden, or, let me add, with the archoplasm which is supposed to form the spindle and astral rays of the dividing cell. The idiosome of Meves was supposed to be a mass of protoplasm surrounding the centrioles in the resting cell. In other words, it derived its essential character from its relation to the centrioles. Where then, it is fair to ask, is the idiosome in the insect spermatocyte? Obviously there is none. Fortunately the spermatids furnish a clue which leads to a logical solution of the whole problem. In the vertebrate and pulmonate spermatid there is seldom, if ever, any relation between the idiosome and the centrioles, but there is a constant relation between the idiosome and the Golgi apparatus, a relation which applies as well to the condition seen in the insects. In the spermatocytes of insects, however, the material of the spermatid "idiosome" is scattered throughout the cytoplasm as a part and parcel of each

Golgi body. In other words the densely staining part of each Golgi body is equivalent to the "Golgi apparatus" of authors, while the non-staining substance associated with it is equivalent to the "idiosome" of authors. If the Golgi bodies of the insect could be aggregated into a single mass such as that in the pulmonate "Nebenkern," then we would have the typical idiosome and the typical Golgi apparatus of so many workers. The insects thus furnish a clue to the whole involved series. The non-staining substance which forms the idiosome is really related not to the centrioles, which may be situated within it at certain times, but to the Golgi apparatus. So far as we know at present this intimate relation seems never to be broken. The idea that the "idiosome" is somehow related to the centrioles seems to have been fostered by the view that "idiosome" and "archoplasm" are related, and that the idiosome could thus share in the formation of the spindle. This is certainly negatived in the Hemiptera where the Golgi bodies have nothing to do with the spindle, and by the further fact that in at least some mammals the idiosome is still intact in the metaphase of division when the spindle is fully formed.

I would suggest therefore that before we theorize further on these most confusing structures, a thorough reexamination of the whole subject be made, using as a working hypothesis the possibility that the Golgi apparatus and the idiosome may be essentially a single structural complex. We know that in some cases at least the mitochondria are composed of two distinct substances, one staining readily by the usual methods, the other little if at all. So too the Golgi complex would seem to be similarly duplex in its structure, composed on the one hand of a readily staining substance (the Golgi apparatus) and on the other of a relatively non-staining material (the idiosomic substance). Should further research bear out this possibility, the confusion in which we have been involved will be dissipated and we shall have laid a firm foundation on which to base the future study of these cytoplasmic structures of the germ cells.

SUMMARY.

1. In *Euschistus* the mitochondrial material of the primary spermatocytes occurs in the form of threads which are definitely oriented toward the centrioles in the prophases of the maturation divisions.

2. Due to this very definite arrangement, the threads are so distributed during the anaphases that each daughter cell contains one half of the mitochondrial material.

3. The mitochondria are not divided autonomously on the spindle but as a result of their position or the mechanical action of the constricting cell wall.

4. The Golgi apparatus of the spermatocytes is extensively developed, occurring in the form of scattered Golgi bodies.

5. During the maturation divisions these bodies undergo a process of autonomous fragmentation into dictyosomes which are arranged in a definite relation to the spindle and are then distributed in equal groups to the daughter cells. The arrangement and distribution of the Golgi elements during division is clearly dependent upon the centrioles.

6. In the spermatids the Golgi elements are condensed into a single body, the acroblast, from which the acrosome of the mature sperm is derived.

7. The remnant of the acroblast (or Golgi remnant) ultimately breaks free from the acrosome and is lost in the cytoplasm of the tail probably taking no further part in the formation of the sperm.

8. The centrioles of the spermatid are, as in most (all?) animals, paired, and in this case remain in the " neck " region of the mature sperm.

On the basis of the facts brought out in this study it is suggested that:

1. The regularity in the division of the cytoplasmic elements of the cell, as illustrated by the mitochondria and Golgi bodies of *Euschistus,* is not the result of chance but is accomplished by a definite mechanism focused in the centrioles, and may perhaps partake of the nature of a meristic, as contrasted to a mass division.

2. The acrosome of the animal sperm is probably universally

formed in connection with the Golgi apparatus, and has nothing whatever to do with spindle fibers or other cell parts. It is to be considered as much a characteristic part of the sperm, having a definite morphological value, as are the nucleus and mitochondria.

3. The idiosome plus the Golgi apparatus of vertebrates and pulmonates is to be considered as the homologue of the scattered Golgi bodies of insects, the idiosomic substance and the Golgi material being in some way intimately related, possibly in the sense of an essentially single cell organ of duplex chemical nature.

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EXPLANATION OF PLATES.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3800 diameters. At so great an enlargement it has of course been necessary to correct the outlines extensively and to add much of the finer detail free hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 2300 diameters. In every case the method employed in the preparation of the original object has been indicated.

A, acrosome

a, acroblast

c, centrioles

ch, chromosomes

G, Golgi remnant
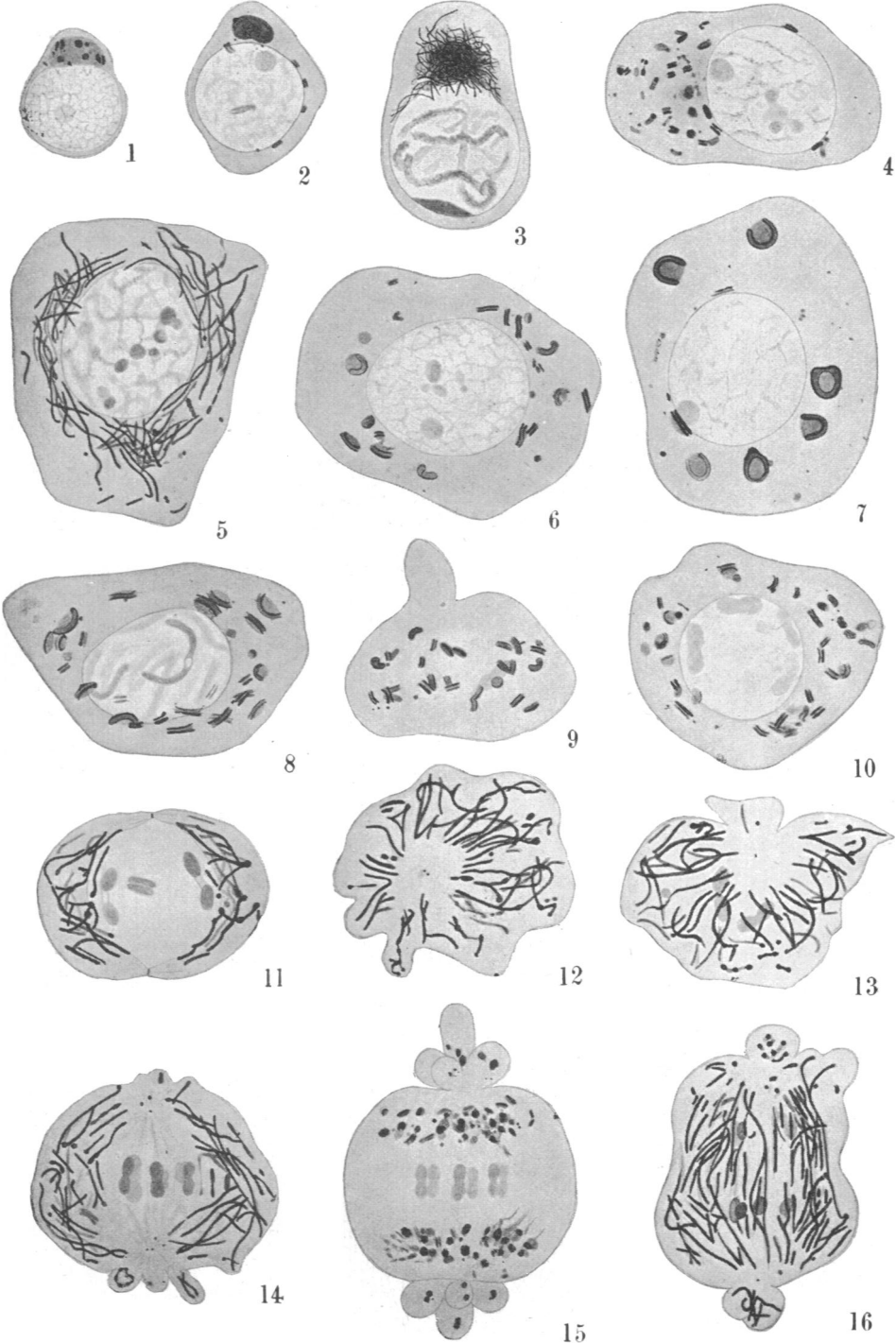
N, nebenkern

n, nucleus

p, pseudo-blepharoplast

PLATE I.

Fig. 30 is from *Euschistus servus;* Figs. 17, 23 and 24 are from *Euschistus euschistoides;* Figs. 18 to 21, 28 and 31 are from *Brochymena quadripustu-lata;* the others are from *Murgantia histrionica.* All the figures are from cells of the small ("normal") generations except 23, 24, 30 and 35.

FIG. 17.   First maturation division telophase.   Division of the mitochondrial "palisade" practically complete.   (Benda.)

FIG. 18.   Second maturation division telophase.   (Modified Kopsch.)

FIGS. 19, 20, 21.   Spermatids.   Progressive stages in the aggregation of the Golgi elements to form the acroblast.   (Modified Kopsch.)

FIG. 22.   Spermatid.   Centrioles double, and the tail filament in first stage of its growth.   (Benda.)

FIG. 23.   Spermatid.   First appearance of the vesicular acrosome within the acroblast.   (Modified Kopsch.)

FIG. 24.   Spermatid.   Later stage in the differentiation of the acrosome. (Modified Kopsch.)

FIG. 25.   Spermatid.   Nebenkern halves elongating; typical granule developed in the acrosome.   (Benda.)

FIG. 26.   Spermatid.   Centrioles in "V" formation.   (Flemming-hematoxylin.)

FIG. 27.   Spermatid.   First steps in formation of swellings on the mitochondrial sheaths.   (Benda.)

FIG. 28.   Spermatid.   Late stage in differentiation of the acrosome.   (Modified Kopsch.)

FIG. 29.   Spermatid.   Pseudo-blepharoplast completely formed; centrioles straightened out along the main axis of the sperm.   (Flemming-hematoxylin.)

FIGS. 30, 31.   Spermatids.   Final steps in separation of the acrosome from the acroblast (Golgi remnant).   (Modified Kopsch.)

FIG. 32.   Spermatid.   Head and portion of the tail with the characteristic swellings on the mitochondrial sheaths.   (Benda.)

FIG. 33.   Immature sperm head.   The chromatin forms a thin axial rod enclosed in a protoplasmic envelop.   (Flemming-hematoxylin.)

FIG. 34.   Mature sperm head from small cell generation.   (Flemming-hematoxylin.)

FIG. 35.   Mature sperm head from large cell generation.   (Hermann-hematoxylin.)